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FINAL REPORT ON

CONTRACT NO DA 92-557-FEC-37969 (No. 1)

INCLUSIVE DATES 15 March 1965 TO 14 March 1966

SUBJECT OF INVESTIGATION

DIFFERENTIATION, CLASSIFICATION
AND
LABORATORY DIAGNOSIS
OF
EL TOR VIBRIOS

RESPONSIBLE INVESTIGATOR

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April, 1966

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ABSTRACT OF THE FINAL REPORT

1. Studies of kappa-type phage

The host range, serological properties and electron microscope morphology of kappa-type phage which is produced specifically by the Celebes type El Tor vibrio were investigated. Kappa-type phage inducing effects of ultraviolet light and mitomycin C were also examined. Lysogenicity of kappa-type phage in El Tor vibrio appeared to be unstable. A kappa-type phage detection method for rapid diagnosis of Celebes type El Tor vibrio carriers was devised.

2. Application of fluorescent antibody technique to cholera studies.

Applicability and limitation of fluorescent antibody technique on diagnosis of cholera vibrio, and cross staining reaction of various vibrios including NAG vibrios and V. parahemolyticus with fluorescent antibody technique were surveyed.

3. Biochemical properties of so-called water vibrios

Biochemical properties of 43 strains of water vibrios were studied and compared with those of known vibrio strains.

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I. PURPOSE OF THE SUBJECT

The purpose of the present study under the Contract No. DA-92-557-FEC-37969 is to clarify the biological properties of El Tor and Asiatic cholera vibrios and apply the results to differentiation, classification and laboratory diagnosis of these vibrios.

The report in the following consists of three different part. The first section describes the properties and practical application of kappa-type phage, which is produced specifically only by the Celebes type El Tor vibrio, and consequently can be used for diagnostic purpose. In the second section several experiments on fluorescent antibody technique are described, which is also considered as useful screening method for early diagnosis of cholera patients or carriers. The last part consists of description of biochemical properties of "vibrios." From the results obtained, it has been concluded that some strains must be excluded from vibrio species.

II. STUDIES OF KAPPA-TYPE PHAGE

1. GENERAL PROPERTIES OF KAPPA-TYPE PHAGE

a. Host Range and Serological Properties: As reported earlier (6), most strains of El Tor vibrios isolated in the epidemics of Southeast Asia produce the specific temperate phage with very narrow host range, and this was tentatively designated as kappa-type phage. Further studies on the lysogenic property of El Tor vibrios (7, 9, 10) have revealed that the temperate phages liberated from three strains isolated in Sarawak in 1961 (SE 1-3) had exceptionally wide host range, and these phages were designated as SE phages. The former was lytic only to Vibrio cholerae strain H218 and a few other strains, while the latter were lytic to 99 of 149 strains of El Tor vibrios and to 22 of 53 strains of V. cholerae. Plaques of all the temperate phages on H218 were turbid, but clear plaque mutant appeared not rarely in the rate of around 10^{-2} to 10^{-3}. The host range of such a mutant was much broader than that of the original phages. All the kappa-type phages were not lytic to H218 mutants resistant to other kappa-type phages or SE phages, while SE phages were lytic to H218 strains resistant to kappa-type phages.

Neutralization test was carried out on various kappa-type and SE phages with rabbit antisera prepared against a kappa-type phage (Itazuke) and a SE phage (SE 3). As shown in Table 1, the neutralization indexes for the anti-kappa-type sera of SE phages were as high as those of kappa-

type phages (90-100), while those for anti-SE 3 phage serum were widely distributed from 30 to 100 irrespective of their types.

From the results above stated, all the temperate phages produced by El Tor vibrios so far tested are considered to be serologically closely related. Therefore, SE phages can possibly be regarded as the host range mutants of kappa-type phage.

The five phages used by Mukerjee (4) for a purpose of phage typing of El Tor vibrios, were also examined for serological properties. All five phages gave the similar results in neutralization test using the sera above stated, and were considered to be closely related with our phages (Table 1).

b. Morphological Properties of Kappa-Type Phage (6, 9, 10): Electron micrographs were prepared by the negative staining technique using sodium phosphotungstate solution as a contrasting material. Both kappa-type and SE phages were indistinguishable in morphology. They were tadpole-like, and had a hexagonal head (about 450-550 A diameter) and tail (about 800-1,000 A length and 150-200 A width) with cross striation. Ghost phages with empty head and contracted tail sheath were frequently observed (Fig. 1).

2. STABILITY OF LYSOGENICITY IN EL TOR VIBRIO STRAINS

It has been already reported that two strains isolated in Djakarta in 1961 (VE 12 and 13) were not lysogenic when received at our laboratory (10). On the other hand, several strains of Celebes type El Tor vibrios have become non-lysogenic during successive cultivations. Moreover, 15 strains isolated during the Celebes type El Tor vibrio epidemics in Philippines and cultivated successively until our investigation on kappa-type phage production, also have been proved non-lysogenic. As shown in Table 2, these organisms were susceptible to kappa-type phage except two strains (VE 12 and 13), and the lysogenized organisms could be isolated from the center of turbid plaque. From these findings, it is probable that the "curing" had occurred naturally in these strains resulting a loss of prophage (13).

Therefore, including the original cultures of such strains, several lysogenic El Tor vibrios were examined on phage-producing ability for isolated single colonies. The results so far obtained were not consistent, but in some strains, the non-lysogenic colonies were obtained in fairly high rate (Table 3). The nature of this phenomenon is still obscure, and further studies are needed for clarification (9, 13).

3. INDUCTION OF KAPPA-TYPE PHAGE

a. Induction by Ultraviolet (UV) Light (10, 13): Inducing effect of UV irradiation for lysogenic El Tor vibrios was examined according to the method of Field and Naylor (2). These phages were easily induced by relatively short time irradiation of UV (15W, 90cm, 15-20sec). Fig. 2 shows an example of UV dosis-yield relationship. The one-step induction curve is shown in Fig. 3. In this experiment, phage titer reached to its maxmum 150 minutes after UV irradiation (90cm, 30sec), and yield was about 10^4-fold of non-induced control. In normal cultures, a ratio of colony formers/plaque formers is around 10^3 as indicated by broken line. The induction rate and burst size calculated from these curves were 96.5% and 150 respectively. As shown in Fig. 2, not production of phage is larger for shorter irradiation, but induction rate was much lower in such a case.

b. Induction by Mitomycin C (MC) (13): Inducing effect of MC was also examined according to the method of Otsuji et al. (5). Appropriate amount of MC was added to the growing culture of Celebes type El Tor vibrio, and the phage titer was measured after 3 hours. As shown in Fig. 4, the optimal concentration was around 0.5 mcg/ml in the shaking culture, but in the stasnding culture, that was only

0.05–0.1 mcg/ml. The turbidity of the growing culture suddenly decreased about 60 minutes after the addition of MC, and this decrease of turbidity reached its maximum within another 60 or 90 minutes (Fig. 5). From the one-step induction curve in Fig. 6, it was shown that relatively small fraction of the population was induced by MC and total yield of phage was only 10^3-fold or the control culture.

4. APPLICATION OF STREPTOMYCIN-RESISTANT INDICATOR STRAIN ON DETECTION OF KAPPA-TYPE PHAGE

Recently we succeeded to get highly resistant (ca. 1 mg/ml) mutant of the indicator strain H218 for streptomycin (SM) by means of direct selection technique. This strain (H218 Sm^r) has the same susceptibility range and E.O.P. value with the original H218 strain (12).

By the use of this strain with SM-containing medium, quantitative phage assay and spot test of diagnostic purpose has been simplified. Even when whole culture of test organism is mixed with the indicator organism, only the free phages can be detected by plaque formation under the presence of SM, and colony formation or phage production after the growth of test organism are inhibited. Consequently, any time-consuming treatment such as centrifugation or filtration is no longer needed for test cultures (13).

5. APPLICATION OF KAPPA-TYPE PHAGE ON EARLY DIAGNOSIS OF CELEBES TYPE EL TOR VIBRIO CARRIERS

As already stated, most strains of Celebes type El Tor vibrio produce the kappa-type phage about 10^{-3} per bacterium. Therefore, after the enrichment culture of fecal specimen, we can detect kappa-type phages simultaneously with the vibrio. Moreover, as phage detection method requires shorter time than ordinary isolation culture technique, this method seems to be convenient as screening diagnosis technique of the carriers of Celebes type El Tor vibrio. In the earlier report (8), the supernatant fluid of the enrichment culture centrifuged at 4,000 rpm for 30 minutes was used for detection of kappa-type phage by means of spotting on a indicator organism overlayed on nutrient agar plate with soft agar. After the establishment of SM-resistant indicator strain, however, the centrifugation of enrichment culture is ommitted, and the culture fluid is directly spotted on the H218 Sm^r mixed with soft agar containing 100 mcg/ml of SM and overlayed on nutrient agar plate (Table 4). This technique has been shown more sensitive than ordinary culture technique (Table 5) and proved very applicable in the field work (13).

III. APPLICATION OF FLUORESCENT ANTIBODY TECHNIQUE TO CHOLERA STUDIES

1. APPLICATION OF FLUORESCENT ANTIBODY TECHNIQUE TO THE DETECTION OF CHOLERA VIBRIO

Applicability and limitation of fluorescent antibody technique on the diagnosis of cholera vibrio were surveyed (14). Fluorescent anti-El Tor vibrio antibody was prepared from hyperimmune rabbit sera according to the method of McDevitt et al. (3). Details of fluorescent microscopy is shown in Table 6. Both El Tor and Asiatic cholera vibrios were stained specifically with anti-El Tor vibrio fluorescent antibody irrespective of their types, whereas laboratory strains of Gram-negative rods other than vibrio species could not be stained with the same antibody solution (Table 7). In the fecal specimens from the non-cholera patients and ...althy persons, positively stained organisms or particles ...re found in a very high rate (88%), in which only a few ...re strongly positive (Table 8). These positively stained ...ganisms or particles were easily differentiated from cholera ...brio in the morphological properties or strength of fluores...ence. The differentiation of specific and non-specific ...tain was not difficult especially with the aid of observation

by dark-field illumination of the same field using the visible light. Fig. 7 shows the differentiation of El Tor vibrio and Escherichia coli by means of such a technique.

The limit of bacterial concentration in this technique to find the stained organisms obviously in the smear was around 10^6/ml, but after the enrichment culture for 6 hours, the vibrios can be detected from the material which initially contained vibrios as few as several hundreds/ml (Table 9). On the whole, this technique is considered as an effective screening method to detect cholera patients or carriers, as this technique requires shorter time than the ordinary isolation technique.

2. STUDIES OF CROSS STAINING REACTION OF VIBRIOS (15)

Various vibrio strains including NAG vibrios and Vibrio parahaemolyticus were stained with the fluorescent anti-El Tor vibrio antibody to examine the presence of cross staining reaction.

Although no cross agglutination existed, the cross staining reaction was observed between cholera vibrios and other vibrio species. Cholera-like NAG vibrios and V. parahaemolyticus gave strongly or moderately positive reaction, whereas other species and so-called water vibrios gave only weakly positive or negative reaction (Table 10). Flagellum

was stained in all the strains of cholera vibrios and V. parahaemolyticus tested, but negative in most other strains.

The NAG vibrio with positive staining reaction (strain 4714) could adsorb only the antibody reacting with its own antigen from the fluorescent antibody solution. On the other hand, cholera vibrios as well as El Tor vibrios could adsorb the whole antibodies from the same solution (Table 11).

The homologous staining reaction was not inhibited by sonication, heating and trypsin treatment, but the staining reaction with heterologous fluorescent antibody was strongly inhibited by such treatments. Treatment with cold or hot trichloracetic acid inhibited both homologous and cross staining reactions. Treatment of acetone-dried organism with 95% phenol at 37 C and trypsinization thereafter, gave strong inhibition of cross staining reaction (Table 12).

The results indicate that only the antigen of O-nature contributes to the specific staining reaction, and the cross reaction of NAG vibrios is due to the common antigen(s) of protein nature. The use of anti-NAG vibrio fluorescent antibody gave the similar results and conclusion.

IV. BIOCHEMICAL PROPERTIES OF SO-CALLED WATER VIBRIOS

As stated in the former section, some strains of so-

called water vibrios gave negative fluorescent antibody staining, whereas most other vibrios gave usually positive reaction. Biochemical properties were examined on these vibrios to clarify the taxonomical relationships between them (11).

Forty-three strains of water vibrio, of which 5 were stock strains and other 38 were newly isolated, 2 strains of V. cholerae, 3 strains of El Tor vibrio, 2 strains of NAG vibrio, and 3 strains of known vibrio species (V. metchnikovii. V. denekei and V. proteus) were tested for the following biochemical characteristics: 1) Acid production from glucose, mannitol, mannose, galactose, fructose, arabinose, rhamnose, sucrose and lactose; 2) Hugh-Leifson test; 3) VP test; 4) MR test; 5) reactions in SIM medium (H_2S, PPA and motility); 6) indole; 7) cholera red; 8) reduction of nitrate; 9) urease; 10) gelatin liquefaction; 11) Kovac's oxidase; 13) cytochrome oxidase; 14) KCN test; 15) utilization of citrate (Simmons); 16) utilization of malonate; 17) lysine decarboxylase; 18) arginine decarboxylase; 19) growth under the presence of 3% or 7% NaCl; 20) susceptibility to triphenyltetrazolium chloride (TTC); 21) susceptibility to vibriostatic agent O/129; 22) fluorescence under UV light.

The results is shown in Table 13. V. cholerae, including El Tor vibrios, NAG vibrios and other vibrios show the similar pattern in this table, but 5 stock strains of

water vibrio seem completely different. According to Davis and Park's criteria (1), especially negative sugar decomposition and resistance to vibriostatic agents, these strains are considered to be "Comamonas" sp.. Two of newly isolated water vibrio showed similar characteristics. On the other hand, we could not get distinct difference between Asiatic cholera vibrios and El Tor vibrios or other vibrio species by the biochemical characteristics so far tested. Differentiation of such vibrios by biochemical method needs further investigation.

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APPENDIX A

Neutralization tests of El Tor vibrio temperate phages

Phages		Neutralization indexes with	
		Anti-kappa type(Itaszuke) phage serum	Anti-SE(3) phage serum
Kappa type phages			
Itaszuke		100	50
Other 35 strains		50 - 100	50 - 100 (mostly 50 - 80)
SE phage			
SE3		99	100
SE1, 2		95 - 100	50 - 100
El Tor vibrio typing phages (Nakarjima)	I	100	97
	II	100	100
	III	100	90
	IV	99	99
	V	100	97

Neutralization index = $(N_1 - N_2) / N_1 \times 100$

N_1 = Number of plaques in a control plate

N_2 = Number of plaque after neutralization

Table 1. Neutralization tests of El Tor vibrio temperate phages

Sensitivity of K type phage-negative strains to K type phage

VE 12	-	61-7891	+
VE 13	-	61-7870	+
VE 126*	+	61-10348	+
PE 1*	+	62-3755	+
PE 4*	+	62-4191	+
PE 9*	+	62-4192	+
PE 20*	+	64-1711	+
Mal 1*	+	64-1751	+
Mal 3*	+	64-1851	+
Mal 4*	+	64-1879	+
JE 186*	+	65-1270	+
		65-1466	+
		65-4382	+

* Lysogenicity was lost during the successive cultivations.

Table 2. Sensitivity of kappa-type phage-negative strains to kappa-type phage

Percentage of lysogenic colonies found at random generations of successive cultures of Celebes type El Tor cholera vibrio

Generations of successive cultures	PE 1		PE 4		PE 9		PE 20		Mal 2	
	P*	A*	P	A	P	A	P	A	P	A
15			100				96		100	
21		100		100		100		88		100
29		100	98	100		100	96	100	98	100
39	96	100	100	100	98	100	98	100	100	99
44	98		100		98		100		97	
58	100	100	100	100	100	96	98	99	98	100

* P = peptone water; A = nutrient agar slant

Table 3, Percentage of lysogenic colonies found at random generations of successive cultures of Celebes type El Tor cholera vibrio

Enrichment culture in alkaline peptone water for 5-10 hours

Isolation of vibrio by ordinary method	"Spot test" on SM-resistant indicator strain H218 SM^r
	Incubation for 8 hours
	Lysis (+)
	Identification must be carried out by neutralization test and/or lysis test using kappa-type phage resistant strain of H218.

Table 4. A schema for routine diagnosis of Celebes type El Tor vibrio carriers

Production of K type phage in peptone water culture of Celebes type El Tor cholera vibrio mixed with human feces

Tube No.		1	2	3
Initial population of vibrio/ml		0.12	1.2	12
3 hrs	No. of PFU/ml	0	10	50
	"Spot test"	-	-	-
	Colonies on TCBS plate	0	0	0
6 hrs	No. of PFU/ml	0	1.4×10^2	2.6×10^3
	"Spot test"	-	+	+
	Colonies on TCBS plate	0	0	52

Table 5. An example of model experiment comparing ordinary isolation technique and kappa-type phage detection technique. In the tube 2, 6 hours after inoculation, only kappa-type phage was detectable

Microscope: Leitz Ortholux

UV-source: OSRAM HBO 200W

Exciter filter: Schott KG1, BG38 and x UG1

Secondary filter: Kenko L40 or Schott UV-abs

Objective: Achromat x 100

Ocular: GF x 10

Camera: Leica If with MIKAS

Film: Fuji SS or Fuji color R (ASA 100)

Exposure: 1 to 2 min for black and white

2 to 3 min for color picture

Table 6. Details of fluorescent microscopy technique

Staining reactions of various Gram-negative bacteria

Species	No. of strains	Number of strains showing fluorescence with the intensity of				
		++++	+++	++	+	-
V. cholerae (Asiatic)	10	7	3	0	0	0
" (El Tor)	17	17	0	0	0	0
V. metschnikovi	1	0	0	1	0	0
V. proteus	1	0	0	1	0	0
V. danubei	1	0	0	1	0	0
NAG vibrio	2	0	1	1	0	0
Water vibrio	5	0	0	0	2	3
V. parahaemolyticus	10	0	0	9	1	0
E. coli	3	0	0	0	1	2
Salm. typhosa	1	0	0	0	0	1
Salm. paratyphi A	1	0	0	0	0	1
Salm. paratyphi B	1	0	0	0	0	1
Salm. typhimurium	2	0	0	0	0	2
Shigella dysenteriae	4	0	0	0	0	4
Shigella flexneri	1	0	0	0	0	1
Proteus vulgaris	3	0	0	0	1	2
Klebsiella pneumoniae	1	0	0	0	0	1
Aerobacter aerogenes	1	0	0	0	0	1
Serratia marcescens	1	0	0	0	1	0
Pseudomonas aeruginosa	1	0	0	0	0	1

Table 7. Staining reactions of various Gram-negative bacteria

Staining reactions of fecal smears from non-cholera patients

No. of cases	Number of smears showing fluorescence* with the intensity of				
	++++	+++	++	+	±~-
34	2	3	20	5	4

* The specimens were classified according to the strongest fluorescence found in the specimen.

Table 8. Staining reactions of fecal smears from non-cholera patients

Fluorescent antibody staining of El Tor cholera vibrio after the multiplication in enrichment culture for 6 hours

Initial population (CFU/ml)	2.75×10^5	2.75×10^4	2.75×10^3	2.75×10^2	2.75×10^1
Number of vibrio in one microscopic field	138	31	3.1	0.5	>1 in whole fields
Number of vibrio in culture (/ml)	7×10^8	1.5×10^8	1.5×10^7	2.5×10^6	?

Table 9. Fluorescent antibody staining of El Tor cholera vibrio after the multiplication in enrichment culture for 6 hours.

Staining reactions of various vibrios with anti-JE5 (El Tor) fluorescent antibody

Strain	Staining reaction		Agglutination reaction
	basillary body	flagellum	
BAO 4714	+++	-	- (<1:20)
4715	++	++	-
Water vibrio			
VV2	-	-	-
VV6	-	-	-
VV8	+	-	-
VV11	±	-	-
VV16	+	-	-
V. parahaemolyticus			
O-1	++	++	-
O-2	++	++	-
O-3	++	+++	-
O-4	++	+	-
O-5	++	+	-
O-6	+	±	-
O-7	++	+	-
O-8	++	++	-
O-9	++	++	-
O-10	++	+++	-
V. metschnikovi	++	-	-
V. proteus	++	-	-
V. denekei	++	-	-

Table 10. Staining reactions of various vibrios with anti-JE5 (Ogawa type El Tor) fluorescent antibody

Staining reaction of vibrios by anti-JE5 (El Tor) fluorescent antibody adsorbed with various antigens

Adsorbing antigen	Stained organism	Staining reaction	
		bacillary body	flagellum
None	JE5	++++	++++
	Ogawa	++++	++++
	4714	+++	-
	O-3	++	+++
	V. matchnikovi	++	-
	V. proteus	++	-
	V. denekei	++	-
JE5	JE5	-	-
	Ogawa	±	±
	4714	±	-
	O-3	±	-
	V. matchnikovi	±	-
	V. proteus	±	-
	V. denekei	±	-
Ogawa	JE5	±	±
	Ogawa	±	±
	4714	±	-
	O-3	±	-
4714	JE5	++++	++++
	4714	±	-
	O-3	±	++
O-3	JE5	++++	++++
	4714	+++	-
	O-3	±	-

Table 11. Staining reaction of vibrios by anti-JE5 (Ogawa type El Tor) fluorescent antibody adsorbed with various antigens

Effect of Various Treatment on FA-stain of El Tor and NAG Vibrios

Treatment	El Tor	NAG
None	++++	+++
Heat (100°C, 10 min)	++++	++
Sonication (10 KC, 15 min)	++++	+++
Sonication and heat	+++	++
Sonication and trypsin (0.25%, 30 min)	+++	++
Sonication, heat and trypsin	+++	+
Hot TCA (4%, 100°C, 10 min)	+++	+
95% Phenol (37°C, 4 days)	++++	±
Phenol and trypsin	+~++	-

Table 12. Effect of various treatment on fluorescent antibody stain of El Tor (Ogawa type) and NAG vibrio

	Water vibrios		V. cholerae* and NAG vibrios	Other vibrio species§
	Stock	Newly isolated		
Glucose	0/5**	36/38	7/7	3/3
Gas from glucose	0/5	0/38	0/7	0/3
Mannitol	0/5	36/38	7/7	1/3
Mannose	0/5	34/38	7/7	3/3
Galactose	0/5	29/38	7/7	3/3
Fructose	0/5	36/38	7/7	3/3
Arabinose	0/5	5/38	0/7	0/3
Rhamnose	0/5	0/38	0/7	0/3
Sucrose	0/5	36/38	7/7	3/3
Lactose	0/5	24/38	7/7	1/3
VP	0/5	36/38	6/7	1/3
MR	0/5	6/38	0/7	0/3
Hugh-Leifson F	0/5	35/38	7/7	3/3
Hugh-Leifson O	1/5	0/38	0/7	0/3
SIM H_2S	1/5	6/38	0/7	0/3
SIM PPA	1/5	0/38	0/7	0/3
SIM motility	5/5	38/38	7/7	3/3
Indole	0/5	13/38	7/7	3/3
Cholera red	0/5	4/38	7/7	0/3
Nitrate	2/5	10/38	7/7	0/3
Urease	0/5	2/38	0/7	0/3
Gelatin	0/5	0/38	7/7	3/3
Catalase	5/5	38/38	6/7	3/3
Kovac's oxidase	5/5	12/38	7/7	2/3
Cytochrome "	5/5	22/38	7/7	3/3
KCN	0/5	6/38	1/7	2/3
Citrate	1/5	27/38	6/7	0/3
Malonate	0/5	0/38	0/7	0/3
Lysine	0/5	21/38	6/7	3/3
Arginine	0/5	9/38	5/7	3/3
NaCl 3%	1/5	38/38	7/7	3/3
NaCl 7%	0/5	14/38	0/7	0/3
TCBS (9,000x)	0/5	3/38	0/7	0/3
O/129	0/5	36/38	7/7	3/3
Fluorescence	0/5	0/38	0/7	0/3

* V. cholerae: M2, M218
El Tor vibrio: JB5, Mal 4, Tor A
NAG vibrio: 4714, 4715
§ V. metschnikovii, V. denokoi, V. proteus
** Number of positive strains/ number of strains tested

Table 13. Biochemical properties of vibrios

APPENDIX B

Figure 1. Electron micrograph of kappa-type phage (Itazuke). A complete phage particle and several ghost phages with contracted tail sheath are seen.

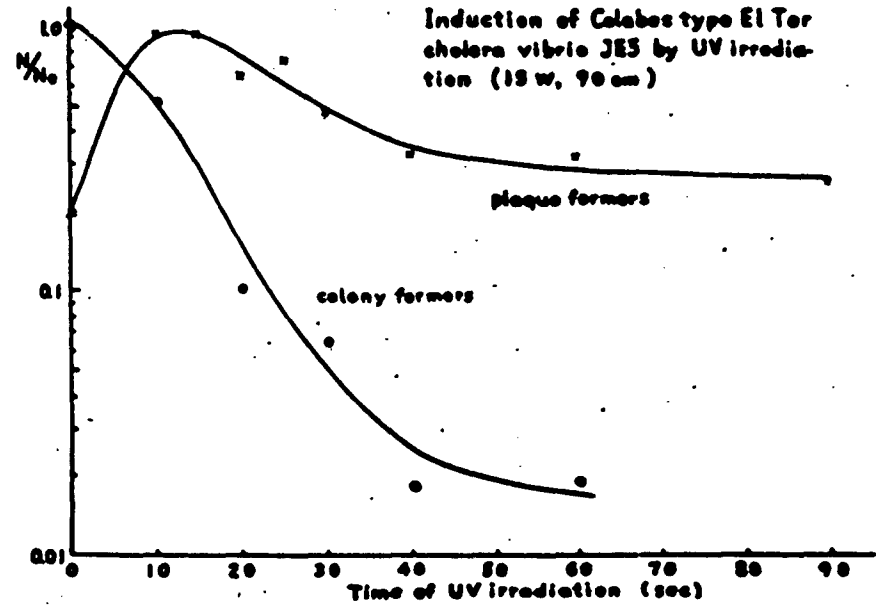


Figure 2. Induction of Celebes type El Tor cholera vibrio JE5 by UV irradiation (15W, 90cm)

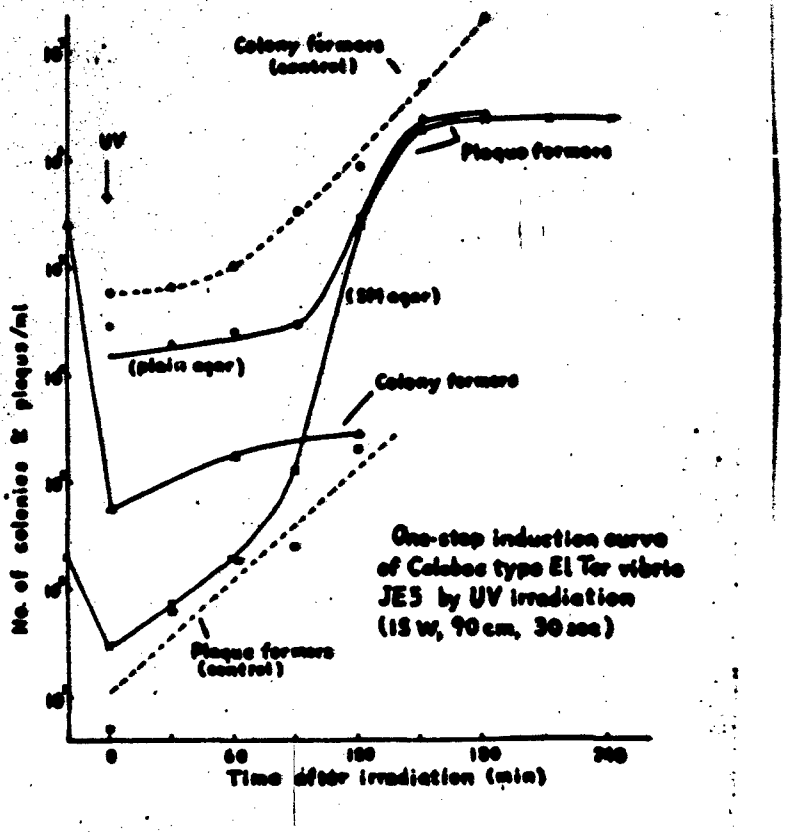
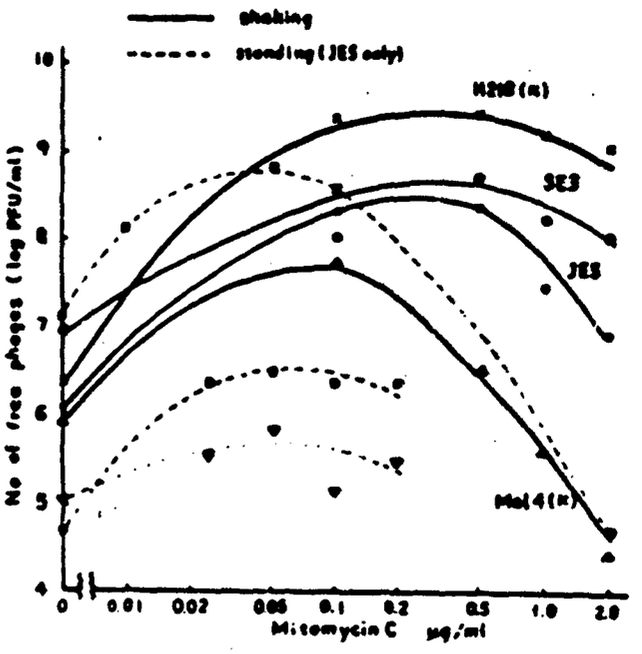


Figure 3. One-step induction curve of Celebes type El Tor vibrio JE5 by UV irradiation (15W, 90cm, 30sec)



Induction of Celebes type El Tor cholera vibrios by mitomycin C

Figure 4. Induction of Celebes type El Tor cholera vibrios by mitomycin C. H218(kappa) and Mal 4(kappa) are strains artificially lysogenized.

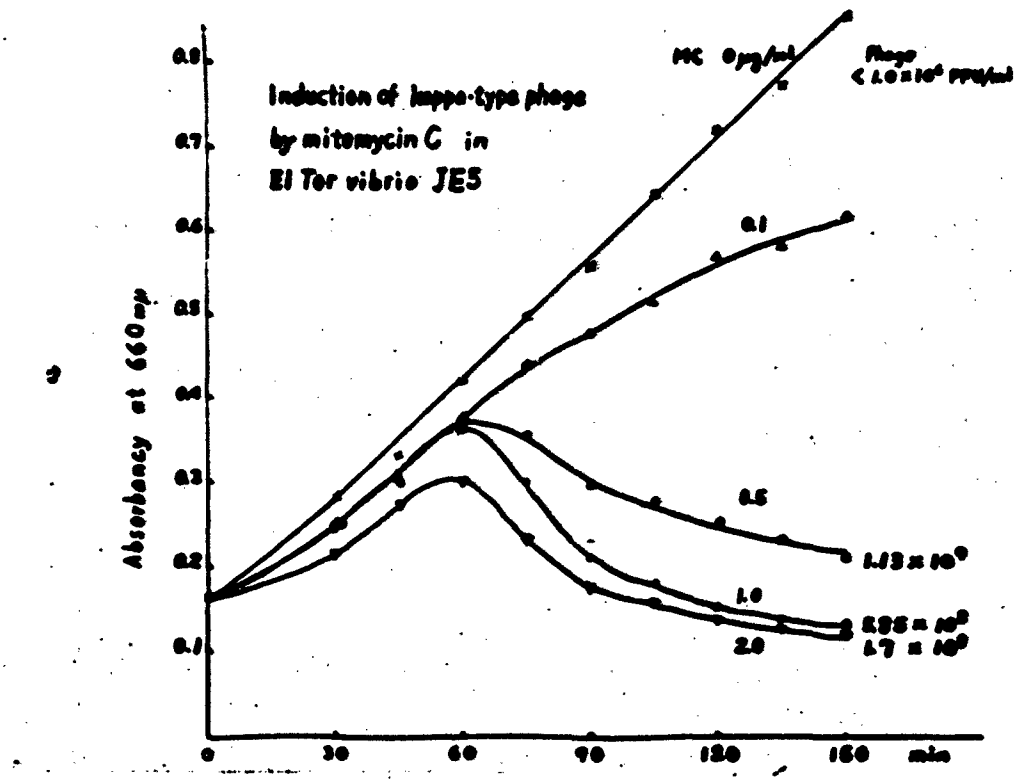


Figure 5. Effect of mitomycin C on the growing culture of Celebes type El Tor cholera vibrio JE5. MC was added at time 0.

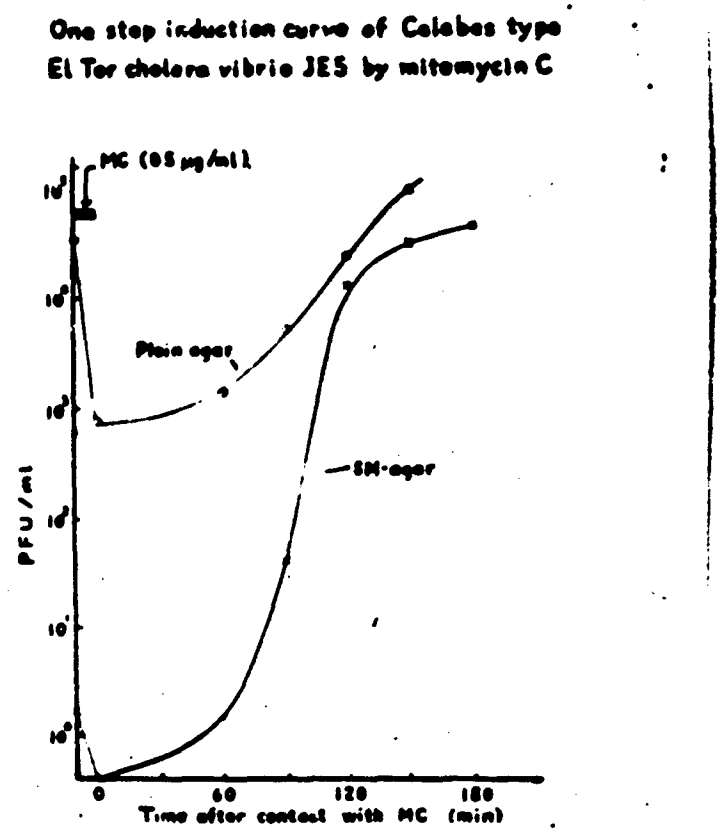


Figure 6. One-step induction curve of Celebes type El Tor cholera vibrio JE5 by mitomycin C.

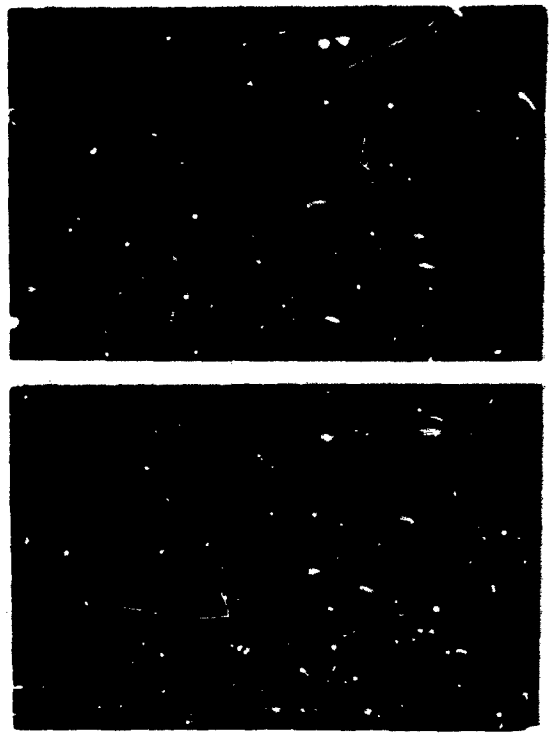


Figure 7. Fluorescent antibody staining of El Tor cholera vibrio mixed with E. coli. By UV illumination, only El Tor cholera vibrios are visible (a). (b) is the same field taken by visible light. E. coli can be seen only in (b). Notice the morphological difference between them.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of Bacteriology
School of Medicine, Kyushu Univ.
Fukuoka, Japan

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
DIFFERENTIATION, CLASSIFICATION AND LABORATORY DIAGNOSIS OF EL TOR VIBRIOS (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final report, 15 March 1965 - 14 March 1966

5. AUTHOR(S) (Last name, first name, initial)
Takeya, Kenji

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
25 May 1966	34	15

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
DA-92-557-FEC-37969	J-231
b. PROJECT NO. 2N014501B71D	
c. Task 00 027FE	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this report from DDC

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U. S. Army R&D Group (Far East) APO San Francisco 96343

13. ABSTRACT

1. Studies of kappa-type phage. The host range, serological properties and electron microscope morphology of kappa-type phage which is produced specifically by the Celebes type El Tor vibrio were investigated. Kappa-type phage inducing effects of ultraviolet light and mitomycin C were also examined. Lysogenicity of kappa-type phage in El Tor vibrio appeared to be unstable. A kappa-type phage detection method for rapid diagnosis of Celebes type El Tor vibrio carriers was deviced.

2. Application of fluorescent antibody technique to cholera studies. Applicability and limitation of fluorescent antibody technique on diagnosis of cholera vibrio, and cross staining reaction of various vibrios including NAG vibrios and V. parahemolyticus with fluorescent antibody technique were surveyed.

3. Biochemical properties of so-called water vibrios. Biochemical properties of 43 strains of water vibrios were studied and compared with those of known vibrio strains. (Author)

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